U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-265 257

Long-Term Effects of Lead Exposure on Three Generations of Brook Trout ('Salvelinus fontinalis')

Environmental Research Lab -Duluth, Minn

9 Mar 76

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/J-76-022	2. JOURNAL ARTICLE
3. PB 265 257	
4. TITLE AND SUBTITLE: LONG-TERM EFFECTS OF LEAD EXPOSURE ON THREE GENERATIONS OF BROOK TROUT (SALVELINUS FONTINALIS)	5. REPORT DATE: May 1976
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): G.W. Holcombe, D.A. Benoit, E.N. Leonard, and J.M. McKim	8. PERFORMING ORGANIZATION REPORT NO.: ERL-DUL-086
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth MN 55804	10. PROGRAM ELEMENT NO.: 1BA608
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS: Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth MN 55804	13. TYPE OF REPORT AND PERIOD COVERED: Journal Article
	14. SPONSORING AGENCY CODE: EPA/600/03

15. SUPPLEMENTARY NOTES: CONTACT: G.W. Holcombe, 8-783-9566 (Duluth, MN)
as published in J. Fish. Res. Board Can. 33(8): 1731-1741.

16. ABSTRACT

Exposure of three generations of brook trout (Salvelinus fontinalis) to mean total lead concentrations (0.9-474 µg/1) showed that all second-generation trout exposed to 235 and 474 µg Pb/1 and 34% of those exposed to 119 µg Pb/1 developed severe spinal deformities (scoliosis). Scoliosis also appeared in 21% of the newly hatched third-generation alevins exposed to 119 µg Pb/1, and weights of these fish 12 wk after hatch were significantly reduced. Gill, liver, and kidney tissues of first- and second-generation brook trout accumulated the greatest amount of lead. Only small amounts accumulated in the edible muscle. An equilibrium of lead residues was reached in liver and kidney tissue from second-generation fish after 70 wk of exposure, but not in gill tissue. Fish exposed to 119 µg Pb/1 and then placed in uncontaminated control water for 12 wk showed a 70, 78, and 74% loss in micrograms Pb per gram for gill, liver and kidney tissue, respectively, and a 39, 56, and 35% loss, respectively, in the total micrograms of Pb in the whole tissue. Residue analysis of eggs, alevins, and juveniles showed that lead was accumulated during these life stages. The maximum acceptable toxicant concentration (MATC) for brook trout in water with a hardness of 44 mg/liter (as $CaCO_3$) and a pH of 6.8-7.6 lies between 59 and 119 µg/liter for total lead and between 39 and 84 µg/liter for dissolved lead. The MATC was based on the development of scoliosis in second- and third-generation fish and the reduced growth of 12-wk-old third-generation trout.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicology, Growth, Lead (metal), Reproduction (Biology), Trout, Survival, Toxicity, Scoliosis, Tissues (Biology), Water Pollution	Brook trout, Residues, Chronic test	06F, 06T

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT: RELEASE TO PUBLIC	19. SECURITY CLASS (This Report): UNCLASSIFIED	21. NO. OF PAGES: 13
	20. SECURITY CLASS (This page): UNCLASSIFIED	22. PRICE: PC A02/MF A01

EPA Form 2220-1 (9-73)

EPA-600/J-76-022
JOURNAL ARTICLE

Long-Term Effects of Lead Exposure on Three Generations of Brook Trout (*Salvelinus fontinalis*)

G. W. Holcombe, D. A. Benoit, E. N. Leonard, and J. M. McKim

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, Minn. 55804, USA

Holcombe, G. W., D. A. Benoit, E. N. Leonard, and J. M. McKim. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 33: 1731–1741. 1976

Exposure of three generations of brook trout (*Salvelinus fontinalis*) to mean total lead concentrations (0.9–474 μg/liter) showed that all second-generation trout exposed to 235 and 474 μg Pb/liter and 34% of those exposed to 119 μg Pb/liter developed severe spinal deformities (scoliosis). Scoliosis also appeared in 21% of the newly hatched third-generation alevins exposed to 119 μg Pb/liter, and weights of these fish 12 wk after hatch were significantly reduced. Gill, liver, and kidney tissues of first- and second-generation brook trout accumulated the greatest amount of lead. Only small amounts accumulated in the edible muscle. An equilibrium of lead residues was reached in liver and kidney tissue from second-generation fish after 70 wk of exposure, but not in gill tissue. Fish exposed to 119 μg Pb/liter and then placed in uncontaminated control water for 12 wk showed a 70, 78, and 74% loss in micrograms Pb per gram for gill, liver, and kidney tissue, respectively, and a 39, 56, and 35% loss, respectively, in the total micrograms of Pb in the whole tissue. Residue analysis of eggs, alevins, and juveniles showed that lead was accumulated during these life stages. The maximum acceptable toxicant concentration (MATC) for brook trout in water with a hardness of 44 mg/liter (as $CaCO_3$) and a pH of 6.8–7.6 lies between 58 and 119 μg/liter for total lead and between 39 and 84 μg/liter for dissolved lead. The MATC was based on the development of scoliosis in second- and third-generation fish and the reduced growth of 12-wk-old third-generation trout. The 96-h LC50 for brook trout was 4100 μg/liter based on total lead and 3362 μg/liter based on dissolved lead; therefore, the application factor (MATC/96-h LC50) lies between 0.012 and 0.029 for both total and dissolved lead.

Holcombe, G. W., D. A. Benoit, E. N. Leonard, and J. M. McKim. 1976. Long-term effects of lead exposure on three generations of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 33: 1731–1741.

Après exposition de trois générations d'ombles de fontaine (*Salvelinus fontinalis*) à des concentrations totales moyennes de plomb (0.9–474 μg/litre), on constate que tous les ombles de deuxième génération exposés à 235 et 474 μg de Pb/litre et 34% de ceux exposés à 119 μg de Pb/litre manifestent de sérieuses déviations de la colonne vertébrale (scoliose). La scoliose apparaît également chez 21% des alevins de troisième génération nouvellement éclos exposés à 119 μg de Pb/litre; le poids de ces derniers est significativement moindre 12 semaines après éclosion. Les tissus des branchies, du foie et du rein des ombles de première et deuxième générations accumulent la plus grande quantité de plomb. Il ne s'accumule que de faibles quantités dans le muscle comestible. Un équilibre des résidus de plomb est atteint dans le tissu hépatique et rénal de poissons de deuxième génération après 70 semaines d'exposition, mais non dans le tissu branchial. Les poissons exposés à 119 μg de Pb/litre et ensuite placés dans une eau témoin non contaminée durant 12 semaines perdent 70, 78 et 74% en microgrammes Pb par gramme du tissu des branchies, du foie et du rein respectivement, et 39, 56 et 35% respectivement en microgrammes totaux de Pb pour le tissu entier. L'analyse des résidus dans les œufs, les alevins et les jeunes démontre que l'accumulation du plomb se produit à ces stades. La concentration toxique acceptable maximum (CTAM) pour l'omble de fontaine dans de l'eau d'une dureté de 44 mg/litre (sous forme de $CaCO_3$) et de pH de 6.8–7.6 se situe entre 58 et 119 μg/litre pour le plomb total et entre 39 et 84 μg/litre pour le plomb dissous. La CTAM est fondée sur le développement de la scoliose chez les poissons de deuxième et de troisième générations, et sur la croissance ralentie d'ombles de troisième génération âges de 12 semaines. La CL50 après 96 h pour l'omble de fontaine est de 4 100 μg/litre sur la base de plomb total et de 3 362 μg/litre sur la base de plomb dissous; cependant, le facteur d'application (CTAM/CL50 après 96 h) se situe entre 0.012 et 0.029 tant pour le plomb total que pour le plomb dissous.

Received March 9, 1976
Accepted May 19, 1976

Reçu le 9 mars 1976
Accepté le 19 mai 1976

Printed in Canada (J4236)
Imprimé au Canada (J4236)

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

CONCENTRATIONS of lead are steadily increasing in rivers, lakes, and oceans (National Academy of Sciences 1972). Lead is a cumulative toxic metal that has no known beneficial or desirable nutritional effect on fish or other animals (McKee and Wolf 1963). Lead accumulates in the bones and tissues of fish and in high enough concentrations can cause anemia (Dawson 1935), impairment of the function of the liver, kidney, and spleen (Haider 1964), spinal deformities (Davies and Everhart 1973), and death (McKee and Wolf 1963).

Little information is available, however, on the chronic or long-term toxicity of lead to fish, other than the work of Davies and Everhart (1973) with rainbow trout, *Salmo gairdneri*. Some field survey investigations have been done on lead residues in fish carcasses (Pakkala et al. 1972) and on lead residues in trout tissues (Pagenkopf and Neuman 1974), but the concentrations of lead to which these fish were exposed were not known. No previous studies are available dealing with chronic lead toxicity to fish and residue accumulation involving several successive generations and all developmental stages.

This study was designed to determine the chronic effects of lead on survival, growth, and reproduction of three generations of brook trout (*Salvelinus fontinalis*) exposed over a 3-yr period, and to ascertain the accumulation rate, distribution, and dissipation of lead residues from the tissue of exposed trout. This work was also designed to determine the sensitivity of the various life stages of the brook trout to lead, to determine the maximum acceptable toxicant concentration (MATC), and to calculate an application factor for lead as described by Mount and Stephan (1967).

Materials and Methods

PHYSICAL AND CHEMICAL

Exposure system — The exposure system consisted of a proportional diluter (Mount and Brungs 1967) that delivered 2 liters of test water per cycle to flow-splitting chambers (Benoit and Puglisi 1973). These chambers divided each of the six diluter concentrations four ways into two duplicate adult spawning tanks and two duplicate larval growth tanks. Larval growth tanks were located directly above and drained into the adult spawning tanks of the same concentration. Test-water flow rates averaged 570 ml/min for the adult spawning tanks and 86 ml/min for the larval growth tanks. Replacement time for 90% of the test water was calculated to be approximately 5 h in the adult spawning tanks and larval growth chambers (Sprague 1969). Adult spawning tanks were 91 × 31 × 38-cm stainless steel tanks with a 31-cm standpipe. Larval rearing facilities consisted of an outer glass tank, 55 × 28 × 15 cm, with an inside tank, 43 × 26 × 15 cm, divided lengthwise into two 13-cm-wide growth chambers. Water depth in each chamber measured 13 cm. All tanks were gently aerated, siphoned Monday through Friday, and scrubbed once a week. All adult spawning tanks were covered with fiberglass mesh screens and concealed by curtains to minimize disturbance. Embryos and alevins up to 4 wk old were protected from direct light by black plastic covers and incubated in nearly complete darkness (0.02 lm). A combination of Sylvania Gro-Lux and Duro-Test Vita-Lite (the U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification) fluorescent lamps were used as the main source of illumination. Fluorescent lamps were used in conjunction with incandescent bulbs wired to a light timer which controlled the photoperiod and an automatic dimmer that simulated sunrise and sunset (Drummond and Dawson 1970). Maximum light intensity was 8.5 lm at the water surface of the adult spawning tanks.

Water characteristics — Water throughout the exposure was obtained directly from Lake Superior. It was passed through an ultraviolet sterilizer to kill >99% of possible pathogens and heated to the test temperature by a hot water heat-exchange system. Seasonally adjusted temperatures ranged from 9 to 15 C throughout the juvenile–adult exposures. Temperatures were maintained at 9 ± 1 C during all spawning, embryo-incubation, and 12-wk posthatch alevin–juvenile growth studies. Total hardness, alkalinity, acidity, and pH determinations were made on all tanks, alternately, one tank a week; dissolved oxygen was measured in a different tank twice a week. Means and range for total hardness, alkalinity, and acidity determinations were 44.3 (42–48), 42.6 (38–45), and 4.1 (1–12) mg/liter as $CaCO_3$, respectively. The pH ranged from 6.8 to 7.6, and the mean and range for dissolved oxygen was 8.5 (4.0–11.9) mg/liter. All water characteristics were measured by methods described by the APHA et al. (1971).

Toxicant solution — Lead nitrate [anhydrous, $Pb(NO_3)_2$, reagent grade] was dissolved in distilled water and introduced to the diluter by a chemical-metering device (Mount and Warner 1965). Lead concentrations (Table 1) were assigned by stratified random assignment to each test tank.

Water samples (25 ml/day) were taken Monday through Friday from each adult spawning tank and larval growth tank with fish present. These water samples were acidified to pH 2.5–3.0 and composited for 2-wk periods. All duplicate tanks were analyzed for lead every 2 wk, providing 60 analyses at each duplicate concentration.

Lead analyses before July 1973 were performed by extraction of the ammonium pyrollidine dithiocarbamate (APDC)-lead complex into methyl isobutyl ketone (MIBK) and subsequent analysis by atomic absorption spectrophotometry (AAS) (Brooks et al. 1967) Calibration curves constructed by the method of known additions of lead to control water were used in the analyses. Differentiation between

TABLE 1. Survival, spawning, and egg production of first- and second-generation brook trout (*Salvelinus fontinalis*) exposed to various concentrations of lead.

	Total lead (μg/liter)											
	472.4[a] ±14.2	474.8 ±16.8	235.0 ±6.5	235.4 ±7.1	118.6 ±4.2	119.8 ±4.0	57.9 ±2.1	57.3 ±2.2	34.1 ±1.4	32.8 ±1.4	0.9 (Control) ±0.2	0.8 (Control) ±0.2
	A[b]	B	A	B	A	B	A	B	A	B	A	B
First generation												
Adult survival during spawning (%) (Oct. 1971–Jan. 1972)	100	60	80[c]	80[c]	100	80[c]	100	100	100	100	60[c]	80[c]
No. mature ♂	2	2	2	2	2	2	1	1	2	2	3	2
No. mature ♀	3	3	3	3	3	3	4	2	3	3	2	3
No. ♀ spawning	2.0	0	1.0	3.0	3.0	2.5	4.0	0[d]	2.5	3.0	1.5	2.5
Mean spawnings/♀	2.0	—	3.0	2.7	2.7	2.4	1.8	0	2.0	3.3	3.3	4.0
Mean viable eggs spawned/♀	367	—	562	548	638	323	350	—	383	611	551	462
Second generation												
Adult survival during spawning (Sept. 1973–Feb. 1974)	0[e]	—	—[f]	—[f]	50[d]	100	100	67[d]	100	100	100	83[d]
No. mature ♂	—	—	—	—	1	2	2	2[g]	2	2	2	2
No. mature ♀	—	—	—	—	5	4	4	3[g]	4	4	4	4
No. ♀ spawning	—	—	—	—	1.5	3.0	2.5	2.0	3.5	4	2.0	2.5
Mean spawnings/♀	—	—	—	—	3.3	1.7	1.6	3.0	3.1	3.0	6	3.2
Mean viable eggs spawned/♀	—	—	—	—	0	411	163	236	264	650	187	403

[a]Mean and 95% confidence interval; [b]A and B represent duplicate adult spawning tanks; [c]Deaths due to fighting; [d]Disease-related deaths; [e]All fish died before reaching sexual maturity; [f]Fish were physically unable to spawn because of severe scoliosis and were therefore terminated before spawning; [g]No successful reproduction occurred in first-generation trout; therefore second-generation spawners from A were divided equally between duplicates at time of spawning.

lead that would pass through a 0.10-μm pore size membrane filter (dissolved lead approximation) and lead present without filtering the samples (total lead approximation) was made by using the APDC–MIBK and AAS method on 14 unacidified "grab" samples from each chronic concentration and from two samples from an acute concentration of 4760 μg total Pb/liter. Mean percentages of dissolved lead and 95% confidence intervals for mean total chronic lead concentrations of 34, 58, 119, 235, and 474 μg/liter were 62 ± 7.1, 68 ± 9.2, 71 ± 7.5, 75 ± 7.3, and 81 ± 6.9, respectively. Mean percentage of dissolved lead for acute samples was 82.

After July 1973 anodic stripping voltammetry (ASV) on acid digested samples (J. Poldoski, Environmental Research Laboratory–Duluth, Duluth, Minn., personal communication) and furnace AAS were used to analyze bioassay water samples. Comparisons between extraction–AAS and these latter two methods for lead analysis showed that the latter methods were faster, more sensitive, and agreed well in accuracy with the extraction method. The accuracy of the furnace AAS method was verified by analyzing reference water samples obtained from the Environmental Protection Agency's Analytical Quality Control Laboratory in Cincinnati, Ohio. Three samples specified to be 26, 92, and 350 μg Pb/liter were found to be 28, 88, and 295μg Pb/liter, respectively, using this method. The furnace AAS method and the ASV method were compared by analyzing eight sets of samples from all concentrations and agreement between these methods was found to be within ± 4% for all lead concentrations.

BIOLOGICAL

This study involved 1) exposure of yearling brook trout through spawning (May 1971–January 1972); 2) exposure of the offspring from the initial parental group through spawning (November 1971–February 1974); and 3) exposure of offspring from the second parental group to the juvenile stage (November 1973–May 1974) (Fig. 1). Biological methods for these three generations will be given separately.

First generation — Yearling through adult spawning — Yearling brook trout (50–70 g) were obtained from the Cedar Island Hatchery in Brule, Wisconsin. Fish were acclimated to laboratory conditions for 4 wk before starting the exposure. The experiment began in May 1971 when 32 fish per duplicate were placed by stratified random assignment in each of the 12 exposure tanks. Sex was determined on all fish in September 1971, and they were separated into groups of males and females. Fish were then thinned randomly to a sex ratio of two males to three females, and one spawning substrate was placed in each adult spawning tank. Spawning commenced in November 1971 and continued until mid-December. Experimental procedures used during spawning and parental termination were the same as those described by McKim and Benoit (1971).

After exposures of 2, 9, 20, and 38 wk, subsamples of 4–10 fish per duplicate were anesthetized with M.S.222 (ethyl *m*-aminobenzoate methanesulphonate). Blood was then drawn with a heparinized syringe by caudal puncture, and gill, liver, kidney, gonad, spleen, and muscle samples were dissected. All tissues were rinsed with distilled water, individually wrapped in cellophane, and frozen along with blood samples for subsequent lead analysis (Leonard 1971). Mean percentage recovery and 95% confidence interval of 34 tissue samples spiked at 20 and 40 μg Pb/liter was 107 ± 11.4%.

Second generation — Embryo through adult spawning — Exposure of the second-generation brook trout through one complete life cycle began with the spawning of the first-generation fish (Fig. 1). Bio-

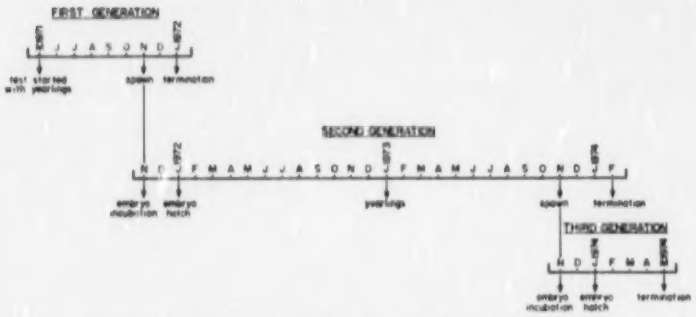

FIG. 1. Chronological order of exposure of three generations of brook trout (*Salvelinus fontinalis*) to lead.

logical methods used during embryo incubation and alevin–juvenile survival and growth studies were the same as those described by McKim and Benoit (1974). Weights were determined on all alevins at hatch and on a subsample of 40 juvenile fish per concentration, 16 wk after hatching. Juveniles were transferred from the larval growth chambers to the adult spawning tanks 12 wk after hatching. All fish were separated according to sex in September 1973 and randomly thinned to a sex ratio of two males to four females in all tanks. Two artificial spawning substrates (Benoit 1974) were placed in each tank, and spawning commenced in November 1973. Experimental methods used during spawning and parental termination were the same as those used for first-generation fish.

Two to five fish from each duplicate tank were randomly selected and anesthetized with M.S. 222 at 46, 70, and 105 wk after hatch. Tissues, including brain, were sampled and frozen for lead analysis as previously described for first-generation fish. Remaining fish in each concentration after 105 wk of exposure were finclipped for identification and transferred to control water. After 12 wk in control water, two fish from each former lead concentration and controls were anesthetized, and gill, liver, and kidney tissues were dissected and analyzed for lead. These tissue samples were used to determine the dissipation of accumulated lead.

Third generation — Embryo through 12-wk-old juveniles — The third-generation exposure began with spawning of the second-generation fish (Fig. 1). This generation was exposed throughout the embryo stage and for 12 wk following hatch. Biological methods were the same as those given for the second-generation alevins except that weights were determined on all surviving juvenile fish 12 wk after hatching.

Ten eggs per duplicate at spawning and two to four alevins per duplicate at hatch and at 1, 2, 4, 8, and 12 wk after hatch were sampled and preserved in 4% formaldehyde solution for lead-residue analysis. Lead residues from alevins preserved in formaldehyde were compared with alevins preserved by freezing at all exposure concentrations and no detectable differences were found between the two methods of preservation.

Trout diets and treatment of diseases and ectoparasites — All fish were fed measured amounts of a commercial trout food (Oregon Moist[1], Glencoe Mills[2], Silver Cup[3], and Ewos[4])[5,6] daily. Food rations were calculated bimonthly by multiplying the total body weight of each individual group of exposed fish by a percentage of body weight ranging from 0.7 to 3.4% depending on the average weight of the fish and water temperature.

Diseases and ectoparasites were treated with neomycin, tetracycline, and formaldehyde as described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Secondary fungal growth (*Saprolegnia*) was controlled by swabbing with a 1% solution of malachite green.

Statistics — Growth and spawning data were subjected to a one-way analysis of variance with an *F*-test of significance ($P \geqslant 0.05$) and subsequent analysis with the Student's *t*-test ($P \geqslant 0.05$) (Steel and Torrie 1960).

Acute toxicity test — A 96-h acute toxicity test was conducted at 12 ± 1 C with 72-wk-old unexposed brook trout (102 g) from the same group used to begin the chronic test. This continuous-flow test with 10 fish per duplicate was conducted according to methods described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

---

[1]Bioproducts, Inc., Warrenton, Oregon 97146

[2]Glencoe Mills, Inc., Glencoe, Minn. 55336, USA.

[3]Murray Elevators, Murray, Utah 84107, USA.

[4]Astra Pharmaceutical Co., Worcester, Massachusetts 01606.

[5]Selection of these commercial diets was based on pesticide content and availability.

[6]Samples from all diets were analyzed for lead and were found to be within the range of 3.6–6.5 μg Pb/g (dry wt).

Results

First Generation

During the 38-wk exposure of brook trout no effects caused by lead on growth, survival, or spawning behavior were observed (Table 1). Means and 95% confidence interval of the lengths and weights of all test fish at thinning were 24.4 ± 1.3 cm and 175.2 ± 27.2 g, respectively. No spawning occurred in one duplicate tank at mean exposure concentrations of 58 and 474 μg Pb/liter; however, spawning did occur in the other duplicates at these concentrations. Mean viable eggs spawned per female at all lead concentrations tested was not significantly different ($P \geq 0.05$) from the controls.

Several yearling trout became hyperactive after 3-wk exposure to 474 μg Pb/liter and would often swim erratically, lose equilibrium, and/or experience muscular spasms when disturbed. Severity of the muscular spasms at this concentration tended to increase with increasing exposure time.

Residue values from the gill, liver, kidney, muscle, spleen, gonads, and red blood cells sampled throughout the 38-wk exposure of trout to 235 μg Pb/liter showed that the kidney accumulated the greatest amount of lead and the gill and liver the next greatest amounts (Fig. 2). Residues from the gill, liver, and kidney tissues of fish from all exposure concentrations were greater than from control tissue. They appeared to reach equilibrium on a micrograms-lead-per-gram basis after 20-wk exposure to 474 and 235 μg Pb/liter, but did not reach equilibrium at lower exposure concentrations (Fig. 3). Spleen and gonads of fish exposed to 235 μg Pb/liter accumulated small amounts of lead (Fig. 2). Muscle tissues from fish exposed to all concentrations accumulated small amounts of lead; however, these levels were not related to exposure concentrations. Red blood cells accumulated lead in direct proportion to increasing concentrations during the first 9 wk of exposure. After 20 wk exposure, residues in the red blood cells from fish exposed to 119, 235, and 474 μg Pb/liter had decreased, and then began to increase and apparently had not reached equilibrium after 38 wk of exposure. On the other hand, red blood cells from fish exposed to 34 and 58 μg Pb/liter appeared to reach equilibrium on a micrograms-per-gram basis after 2 wk (Fig. 3).

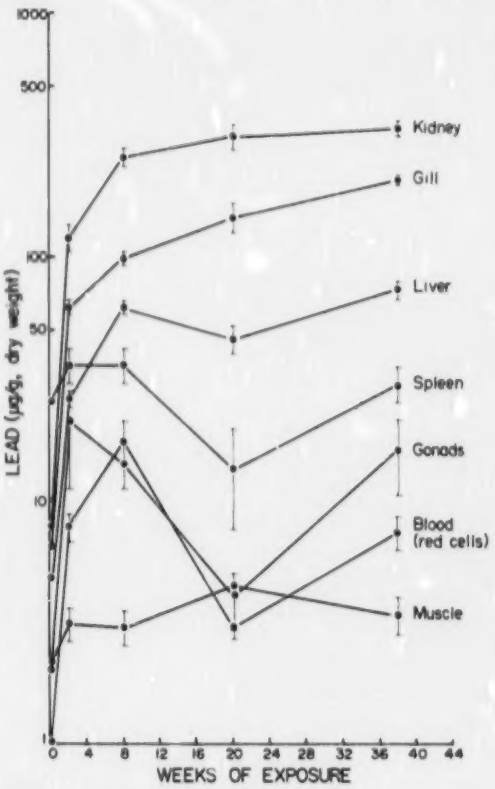

FIG. 2. Lead residues (micrograms per gram dry weight) from various tissues of first-generation brook trout exposed to 235 μg Pb/liter. (●), represents the mean and SE of 10 samples.

Second Generation

Percentages of alevins at hatch with spinal deformities (scoliosis) were 58, 21, 5, 9, 6, and 1, respectively, at concentrations of 474, 235, 119, 58, 34, and 0.9 μg Pb/liter (control). Each alevin hatching with scoliosis was counted as an unsuccessful hatch and discarded. Therefore, because of the large number of spinal deformities, mean hatchability of embryos exposed to 474 and 235 μg Pb/liter was reduced to 28 and 64%, respectively (Table 2). Alevin–juvenile survival and growth after 16-wk exposure to 474 μg Pb/liter were significantly reduced ($P \geq 0.05$) (Table 2). Growth of 65-wk-old fish exposed to 235 μg Pb/liter was also reduced; however, these severely deformed fish were terminated before final growth measurements were taken. There were no effects on growth or survival of fish throughout the entire exposure period at 119 μg Pb/liter and below. Means and 95% confidence interval of lengths and weights of these fish at thinning were 28.5 ± 3.7 cm and 312 ± 66 g, respectively.

Estimated number of eggs per female was multi-

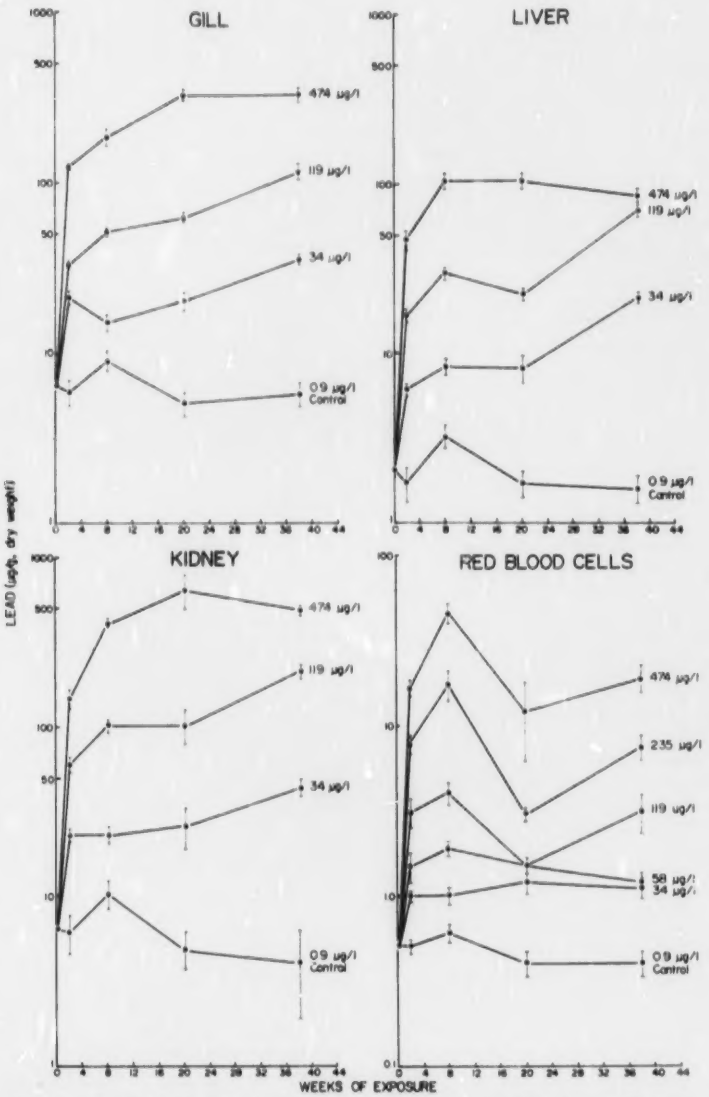

FIG. 3. Lead residues (micrograms per gram dry weight) from gill, liver, kidney, and red blood cells of first-generation brook trout exposed to various concentrations of lead. (●), represents the mean and SE of 10 samples.

plied in succession by percent hatchability, percent alevin–juvenile survival, and individual juvenile weights to yield an estimated total weight of 16-wk-old young per female at each exposure concentration. Combined reduction in hatchability, survival, and growth at 474 µg Pb/liter caused the estimated total weight of 16-wk-old fish produced per female to be significantly lower ($P \geqslant 0.05$) (Table 2). Estimated total weight of 16-wk-old young per female from all lead concentrations was divided by estimated total weight of 16-wk-old young per female in the controls

TABLE 2. Estimated total weight of second- (16-wk-old) and third- (12-wk-old) generation brook trout produced per female at various lead concentrations.

Mean total Pb concn (μg/liter)	Duplicates	Estimated[a] eggs/female	×	Hatchability (%)	×	Alevin-juvenile survival (%)[b]	×	Individual juvenile wt (g)	=	Estimated total wt of young/female (g)	Ratio estimated total wt young/female (exposed : control)
Second generation											
474	A	262		28(4)[d]		20		2.1[e] ± 0.18[e](7)[f]		30.8	0.03
	B	—[g]		—		—		—		—	
235	A	401		73(3)		92		3.4 ± 0.08(20)		915.66	0.81
	B	391		60(8)		96		3.4 ± 0.38(20)		765.73	
119	A	456		86(7)		100		3.1 ± 0.16(20)		1,215.69	0.84
	B	231		85(6)		98		2.7 ± 0.50(20)		519.54	
58	A	250		79(7)		90		3.8 ± 0.33(20)		675.45	0.65
	B	—[h]		—		—		—		—	
34	A	274		57(5)		90		2.8 ± 0.06(20)		393.57	0.75
	B	436		95(9)		82		3.4 ± 0.05(20)		1,154.79	
0.9	A	394		100(5)		84		3.6 ± 0.44(20)		1,191.46	1.00
(Control)	B	330		96(6)		66		4.2 ± 0.11(20)		878.17	
Third generation											
119	A	—[i]		—		—		—		—	0.43
	B	270		70(4)		96		0.88[e] ± 0.07(41)		159.67	
58	A	106		96(2)		98		1.33 ± 0.09(44)		132.63	0.83
	B	155		81(3)		94		1.47 ± 0.05(40)		173.49	
34	A	173		86(3)		92		1.16 ± 0.11(42)		158.78	1.56
	B	427		94(6)[j]		90		1.18 ± 0.10(40)		419.04	
0.9	A	123		86(1)		94		1.27 ± 0.09(44)		126.28	1.00
(Control)	B	265		84(4)		94		1.17 ± 0.15(15)		244.82	

[a]Number of viable eggs produced per spawning female × the probability of spawning (i.e. total spawners/total females = 0.7143 for second and 0.6562 for third generation); [b]Fifty fish exposed in each duplicate; [c]No viable eggs produced; [d]Number of spawnings checked for hatchability; [e]Significantly less than controls ($P = 0.05$, analysis of variance with t-test); [f]Mean and 95% confidence interval; [g]Number of fish sampled; [h]Disease problem; [i]Parental fish suffered moderate scoliosis; therefore, no viable eggs were produced.

FIG. 4. Deformed 65-wk-old second-generation brook trout exposed to 119 μg Pb/liter.

to yield a ratio which was used to compare estimated total weight produced at each lead concentration to the controls (Table 2). This ratio was lower than the control ratio at all exposure concentrations, but the only marked decrease below 50% of the control ratio was at 474 μg Pb/liter.

Hyperactivity, similar to that observed in first-generation brook trout exposed to 474 μg Pb/liter, was first noted in the offspring 8 wk after hatch at this concentration. When disturbed, these juveniles swam erratically and often underwent muscular spasms that appeared to leave them paralyzed for several minutes.

Scoliosis developed in 33% of the alevins that appeared normal at hatch and 39% of all juveniles exposed to 474 μg Pb/liter before 100% mortality occurred at this concentration in December 1972 after 46 wk of exposure. Blackening of the caudal peduncle area of several juvenile brook trout exposed to 235 and 119 μg Pb/liter was first observed after 15 and 27 wk, respectively. All trout with black tails exposed to these concentrations gradually developed scoliosis, which continued to become more severe with longer exposure time. After 65 wk of exposure to 235 and 119 μg Pb/liter, the percentages of fish with scoliosis were 100 and 34, respectively (Fig. 4). Scoliosis was so severe at 235 μg Pb/liter that all fish were physically unable to spawn and were therefore terminated 65 wk after hatch. Fish exposed to 58 μg Pb/liter and below were not affected with blacktail or scoliosis throughout the exposure period.

Spawning occurred at mean lead concentrations of 119, 58, and 34 μg/liter and in the control, and the number of viable eggs produced per female was not significantly different ($P \geq 0.05$) between exposures and control (Table 1). However, no viable eggs were produced in one duplicate tank at 119 μg Pb/liter, because three out of the four females in this duplicate suffered from moderate scoliosis which severely limited their spawning mobility and agility.

Lead-residue uptake and accumulation trends in gill, liver, kidney, muscle, spleen, and gonad tissues of trout were quite similar to residues de-

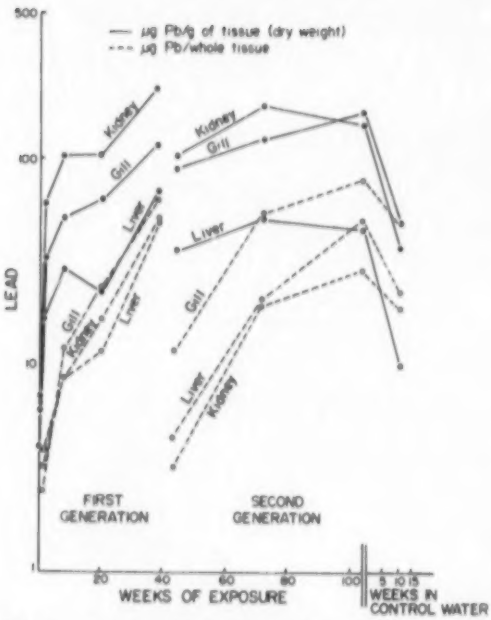

FIG. 5. Lead residues from tissues of first- and second-generation brook trout exposed to 119 μg Pb/liter, and dissipation of residues from tissues of second-generation trout placed in control water. (●), represents the mean of 10 samples.

scribed earlier for first-generation trout. Lead residues (micrograms) per gram of tissue (dry weight) and the lead residue in total tissue from the gill, liver, and kidney of first- and second-generation brook trout exposed to 119 μg Pb/liter are shown in Fig. 5. Total lead present in the gill, liver, and kidney of second-generation fish continued to increase as the fish increased in size. Lead residues per gram of tissue in liver and kidney samples tended to level off and apparently reach an equilibrium but, lead residues per gram of gill tissue continued to increase throughout the exposure and no equilibrium level was achieved. Mean average lead residue values for all muscle tissue sampled from second-generation fish exposed to 119 μg/liter and from control fish were 2.1 and 1.1 μg Pb/g, respectively. Brain tissues did not appear to accumulate lead residues at any exposure concentration.

Gill, liver, and kidney tissues dissected from trout that were exposed to 119 μg Pb/liter for 105 wk and then placed in control water for 12 wk showed a reduction of 70%, 78%, and 74%, respectively, in micrograms of lead per gram of tissue (Fig. 5). Total amount of lead in these tissues was reduced by 39%, 56%, and 35%, respectively (Fig. 5).

THIRD GENERATION

Exposure to 119 μg Pb/liter reduced the hatchability of embryos (Table 2). This reduction was due to the large number of alevins with scoliosis, each of which was counted as an unsuccessful hatch and discarded. Percentage of alevins hatching with scoliosis at 119 μg Pb/liter was 21, whereas 2% hatched with scoliosis at 58 and 34 μg Pb/liter and in the control. Juvenile weights at 12 wk were significantly lower ($P \geq 0.05$) at 119 μg Pb/liter (Table 2). Survival after 12 wk exposure was not affected by lead. The ratio of estimated total weight of fish produced per female was 57% lower than the control ratio at 119 μg Pb/liter and was 56% higher than the control ratio for fish exposed to 34 μg Pb/liter (Table 2).

Subsamples of eggs at spawning and alevins 4 wk after hatch showed that lead residues increased with higher exposure concentrations (Table 3). Embryos contained more lead at each higher exposure concentration. Samples of newly hatched alevins showed negligible lead residues, however, which indicated that most of the lead residues present were contained in the egg membrane and were not accumulated in the embryo. Lead residues in third-generation alevins and juveniles accumulated steadily with increasing exposure time and with increasing lead concentrations throughout the 8 wk following hatch and then appeared to decrease 12 wk after hatch.

ACUTE TOXICITY

The 96-h LC50 of the continuous-flow acute test conducted at 12 ± 1 C on 72-wk-old brook trout was calculated by graphical extrapolation on semilogarithmic paper to be 4100 μg Pb/liter (based on total lead).

MATC AND APPLICATION FACTORS

Effects of lead on survival, growth, and reproduction of brook trout in water with a hardness of 44 mg/liter (as $CaCO_3$) and a pH of 6.8–7.6 demonstrated that the maximum acceptable toxicant concentration (MATC) for this species lies between 58 and 119 μg Pb/liter (based on total lead). The application factor (MATC/96-h LC50) based on total lead lies between 0.014 and 0.029. The MATC and 96-h LC50 values determined using total lead measurements were multiplied by the percentage of filterable lead found to be present at these lead concentrations to obtain a dissolved lead approximation. This multiplication

TABLE 3. Lead residues (micrograms per gram) of third-generation eggs and alevin-juveniles exposed to various water concentrations of lead.

Mean total Pb concn in water (μg/liter)	Eggs at spawn	Wk alevin–juvenile exposed to Pb						
		Hatch 0	1	2	3	4	8	12
119	2.01[a]	0.16 (0.0151)[b]	3.6 (0.0139)	9.6 (0.0124)	9.4 (0.0116)	33.2 (0.0098)	43.8 (0.0427)	20.1 (0.2227)
58	1.70	0.16 (0.0160)	0.16 (0.0159)	0.18 (0.0146)	3.7 (0.0131)	20.1 (0.0121)	25.3 (0.0605)	12.7 (0.2375)
34	0.89	0.18 (0.0143)	0.19 (0.0138)	0.25 (0.0123)	3.40 (0.0113)	10.0 (0.0111)	11.7 (0.0610)	8.0 (0.1776)
0.9 (Control)	0.25	0.19 (0.0136)	0.20 (0.0129)	1.29 (0.0121)	0.24 (0.0112)	0.25 (0.0100)	0.06 (0.0348)	0.36 (0.2287)

[a]Each value represents an average of 20 eggs analyzed.
[b]Mean dry weight in grams of three fish analyzed.

yielded an MATC between 39 and 84 μg/liter based on dissolved lead and a 96-h LC50 of 3362 μg/liter for dissolved lead. The application factor based on dissolved lead lies between 0.012 and 0.025.

Discussion

Results of this study indicated that concentrations of 119 μg Pb/liter and above have an adverse effect on brook trout exposed through several successive generations. The embryo, alevin, and early juvenile life stages of brook trout apparently are more sensitive to lead than later stages in the life cycle. Blackened caudal peduncles, which occurred in second-generation juveniles after 15–27-wk exposure to 119 μg Pb/liter and higher, always preceded the development of scoliosis. In some cases the blacktail condition disappeared after severe scoliosis had developed. Davies and Everhart (1973) also found the alevin and juvenile life stages of rainbow trout to be more sensitive to lead than adults and reported similar occurrences of blacktail and scoliosis in juvenile fish. Haider (1964) produced a blackened caudal region in rainbow trout by injecting lead into the top of the pelvic fins. He also found that trout given lead in their daily food became darkened. The present study showed that spinal curvatures observed in brook trout, which were generally bent toward the left, originated in the region just posterior to the dorsal fin in the same area associated with the blacktail condition (Fig. 4). When brook trout which had recently developed scoliosis were anesthetized in M.S. 222 during periods of blood and tissue sampling, their muscles relaxed and the fish straightened out. Spinal curvatures returned, however, when they were revived. This response indicated that lead was affecting the muscles of the caudal region. Fish that had scoliosis for several weeks did not straighten out under anesthesia, indicating that their spines had possibly become calcified in this deformed position. A biochemical inhibition of metabolism could be responsible for the blacktail and spinal curvature symptoms in brook trout. Nutritional deficiencies of the amino acid tryptophan and the vitamin ascorbic acid have also been found to cause scoliosis and lordosis in trout (National Academy of Sciences 1973). Fati (1961) suggested that lead interferes with the enzymatic degradation of tryptophan, and Pakkala et al. (1972) stated that lead could be a possible enzyme inhibitor because of its affinity for amino, imino, and sulfhydryl groups which comprise reactive sites on enzymes.

Hyperactivity, erratic swimming, muscular spasms, and loss of equilibrium noted in brook trout exposed to 474 μg/liter of lead suggested that lead was affecting the nervous systems of these fish. Haider (1964) obtained similar symptoms of erratic swimming, wave-type muscular movements, and loss of equilibrium in rainbow trout exposed to lead. Previous work has also shown that lead affects the nervous systems of human beings and frogs (Chisholm 1971; Manalis and Cooper 1973).

Since lead residues in liver and kidney apparently reached an equilibrium, analysis of these tisues might be useful in predicting whether or not wild trout populations are being chronically subjected to sublethal lead concentrations that could cause potential damage to the population. Maximum mean residue concentrations for liver and kidney samples of first- and second-generation fish exposed to 119 μg Pb/liter were 68 and 50 μg Pb/g of liver tissue, and 215 and 179 μg Pb/g of kidney tissue, respectively. Lead residues in wild

trout approximating these concentrations in liver and kidney tissue could be detrimental and/or indicate detrimental exposures, since spinal deformities occurred in some laboratory trout with tissue residues of this magnitude and above. Lead residues in gill tissue did not reach an equilibrium according to the micrograms of lead present in a gram of tissue, but tended to increase with continuing exposure (Fig. 5). Similar results were obtained by Pagenkopf and Neuman (1974) with gill and liver tissue from brook trout exposed to 2.81 μg Pb/liter. They found that on a microgram-per-gram basis lead residues in gill tissue continued to increase with age of the fish, whereas liver residues were highest between 1 and 2 yr of age and appeared to reach equilibrium and remain fairly constant in the older fish. Muscle tissue sampled from fish in the present study did not appear to accumulate lead to any substantial degree. Pakkala et al. (1972) analyzed 419 beheaded and eviscerated fish of various species from 17 locations in New York State and also found lead residues to be quite low (range = 0.3–3.0 μg Pb/g) in these samples, which represent primarily muscle tissue. Gonad tissue from first-generation fish exposed to 235 and 474 μg Pb/liter and second-generation fish exposed to 119 μg Pb/liter in the present study had accumulated lead residues before spawning that were higher than control residue levels. After spawning, gonad tissues appeared to accumulate small quantities of lead, but the gonads at this time were in a state of atrophy and the decrease in tissue weight probably accounted for the apparent increase in residues.

Dissipation of lead from tissue of exposed brook trout that were transferred to control water for up to 12 wk indicated that lead was excreted to some degree. After 2 wk in control water, lead was apparently being relocated in the fish. Gill and kidney tissues were losing lead, while liver tissue was further accumulating lead. After 12 wk in control water, however, the gill, kidney, and liver tissues all contained significantly lower lead residues than initially.

The greatest increase in lead residues in third-generation alevins corresponded to the interval when yolk-sac absorption was complete and alevins began to feed (Table 3). Alevins were losing weight and beginning to use their gills during yolk-sac absorption. Rapid uptake of lead through the gills combined with loss of weight probably accounts in part for the high microgram-per-gram lead residue values 4 wk after hatch. Total microgram uptake of lead continued to increase in juveniles at 12-wk exposure, but the microgram-per-gram uptake of lead appears to have decreased. This was probably caused by the rapid growth during this period.

The application factors for lead-exposed brook trout in Lake Superior's soft water lies between 0.014 and 0.029 for total lead and 0.012 and 0.025 for dissolved lead. The application factor calculated using total lead is close to the application factor calculated using dissolved lead. Goettl et al. (1974) obtained an application factor for rainbow trout exposed to lead in hard water of 0.0002–0.0008 when total lead present was used in the calculations, but an application factor of 0.01–0.02 when concentrations of dissolved lead were used. They also calculated an application factor based on the total lead present of 0.006–0.012 with rainbow trout exposed to lead in soft water (28 mg/liter $CaCO_3$). Considering inherent biological variation between groups of fish, the agreement between the application factors calculated for this study and the application factors calculated for rainbow trout exposed to lead in hard and soft water are good, if the hard water application factor calculated on dissolved lead is used.

Acknowledgments

We thank D. L. Olson, B. A. Brakken, and L. H. Mueller for routine water analyses and daily assistance; E. P. Hunt and G. M. Christensen for assistance with biological and biochemical aspects; J. T. Fiandt for chemical and J. H. Tucker for photographic and bacteriological support; T. Thorslund for statistical advice; B. J. Halligan for drafting figures; and other members of the Environmental Research Laboratory-Duluth staff for advice and critical review of the manuscript.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1971. Standard methods for the examination of water and waste water. 13th ed. New York, N.Y. 874 p.

Benoit, D. A. 1974. Artificial laboratory spawning substrate for brook trout (*Salvelinus fontinalis* Mitchill). Trans. Am. Fish. Soc. 103: 144–145.

Benoit, D. A., and F. A. Puglisi. 1973. A simplified flow-splitting chamber and siphon for proportional diluters. Water Res. 7: 1915–1916.

Brooks, R. R., R. J. Presley, and I. R. Kaplan. 1967. APDC–MIBK extraction system for the determination of trace elements in saline waters by atomic absorption spectrophotometry. Talanta 14: 809–816.

Chisolm, J. J. Jr. 1971. Lead poisoning. Sci. Am. 224: 15–23.

Davies, P. H., and W. H. Everhart. 1973. Effects of chemical variations in aquatic environments: III. Lead toxicity to rainbow trout and testing application factor concept. U.S. Environ. Prot. Agency, Water Pollut. Control Res. Ser. 18050 DYC. EPA-R3-73-011c. Washington, D.C. 89 p.

DAWSON, A. B. 1935. The hemopoietic response in the catfish, *Ameiurus nebulosus*, to chronic lead poisoning. Biol. Bull. 68: 335–346.

DRUMMOND, R. A., AND W. F. DAWSON. 1970. An inexpensive method for simulating diel patterns of lighting in the laboratory. Trans. Am. Fish. Soc. 99: 434–435.

FATI, S. 1961. The metabolism of tryptophan in experimental lead toxicity. Biochim. Appl. 8: 280–293.

GOETTL, J. P. JR., J. R. SINLEY, AND P. H. DAVIES. 1974. Water pollution studies. Colo. Div. Wildl. Dep. Nat. Resour. Proj. F-33-R-9. p. 12–36.

HAIDER, G. 1964. Studies on the heavy metal poisoning of fishes I. Lead poisoning of rainbow trout (*Salmo gairdneri* Rich.). Z. Angew. Zool. 51: 347–366.

LEONARD, E. N. 1971. The determination of copper in fish tissues by atomic absorption spectrophotometry. At. Absorpt. Newsl. 10: 84–85.

MANALIS, R. S., AND G. P. COOPER. 1973. Presynaptic and postsynaptic effects of lead at the frog neuromuscular junction. Nature 243: 354–356.

MCKEE, J. E., AND H. W. WOLF. 1963. Water quality criteria. 2nd ed. Resour. Agency Calif., State Water Quality Control Board Publ. 3-A: 206–210.

MCKIM, J. M., AND D. A. BENOIT. 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 28: 655–662.

1974. Duration of toxicity tests for establishing "no effect" concentrations for copper with brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 31: 449–452.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–29.

MOUNT, D. I., AND C. E. STEPHAN. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96: 185–193.

MOUNT, D. I., AND R. E. WARNER. 1965. A serial-dilution apparatus for continuous delivery of various concentrations of materials in water. U.S.H.E.W., Div. Water Supply Pollut. Control. 16 p.

NATIONAL ACADEMY OF SCIENCES. 1972. Committee on biologic effects of atmospheric pollutants. Lead: airborne lead in perspective. Washington, D.C. p. 5–9.

1973. Subcommittee on fish nutrition, committee on animal nutrition, agricultural board. Nutrient requirements of trout, salmon, and catfish. Washington, D.C. 57 p.

PAGENKOPF, G. K., AND D. R. NEUMAN. 1974. Lead concentrations in native trout. Bull. Environ. Contam. Toxicol. 12: 70–75.

[illegible]KKALA, I. S., M. N. WHITE, G. E. BRUDICK, E. J. HARRIS, AND D. J. LISK. 1972. Residues in fish, wildlife, and estuaries. Pestic. Monit. J. 5: 348–355.

SPRAGUE, J. G. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics with special reference to biological sciences. McGraw-Hill, Inc., New York, N.Y. 481 p.

THE COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environ. Prot. Agency, Duluth, Minn., Ecol. Res. Ser. EPA-660/3-75-009. 67 p.